TOXICITY STUDIES ON SELENIUM DISULFIDE SUSPENSION*

EDWARD M. SHAPIRO, M.D., CHARLES M. POMERAT, PH.D. AND J. FRED MULLINS, M.D.

The element selenium, discovered by Berzelius in the early nineteenth century, has had an extremely widespread use in industry. Some of the processes in which selenium plays a part are the coloring of paints and inks, alloying of metals, vulcanization of rubber, and fireproofing of photoelectrical devices. Its most recent use has been in medicine as a sulfide suspension to be applied topically to the scalp as a new treatment for seborrheic dermatitis.

Because of its proximity to arsenic in the periodic table, selenium has long had the reputation for having similar properties and similar toxicity. It is the purpose of this paper to review the toxicity of selenium as well as its mode of action. The toxicity of the selenium suspension is compared to the suspending vehicle alone by tissue culture methods.

Interest in the toxicity of selenium for animals was first aroused when Robinson (17) discovered that the disease of cattle known as blind staggers or alkali disease was caused by the ingestion of selenium in plants upon which they grazed which were grown in a highly seleniferous soil. The signs of selenium poisoning in livestock are loss of weight, emaciation, and loss of hair. The hooves slough and the animals lose control of their voluntary muscles. This results in a staggering gait; hence the name "blind staggers." After it was found that plants grown in the Great Plains region, including South Dakota, Wyoming, and Nebraska, contained a significant quantity of selenium and that animals ingesting these plants developed toxic effects, a concerted effort was made to see whether or not the humans living in those areas might not also be affected.

Smith, Franke and Westfall (25) surveyed 111 families and found no symptoms which they felt were indicative of selenium intoxication. However, in a series of 100 cases with increased selenium in the serum and increased urinary excretion, Smith and Westfall (26) reported 31% possible gastric or intestinal dysfunction and 33% icterus or a past history of jaundice. Lemley and Merryman (8) reported 30 cases of what they considered to be selenium poisoning in humans. In some cases they found the serum selenium levels to be as high as 55 to 600 parts per billion. The symptoms found consisted of depression, dizziness, and clouding of the sensorium. Several people complained of lassitude within three to four hours after awaking in the morning. Intestinal symptoms were frequently present. These consisted of burning epigastric pain, gas and diarrhea. Dermatitis was also seen as a perifollicular, pustular eruption with consecutive brownish, bronzed pigmentation distributed particularly over the hairy surface of the forearms and anterior of the thighs.

Other evidence of toxicity to selenium has been reported as found in industry. Buchan (1) in a review of the literature cites cases with such other symptoms as nasopharyngeal and bronchial irritation, also a persisting garlic-like odor of breath. In this article he cites Dudley (2), who reported eighteen copper refinery workers who had gastric symptoms, increased nervousness and a garlic-like odor of the breath after contact of selenium containing compounds with the skin. One case of a contact dermatitis of the lips following the use of a selenium red lipstick is also reported.

Pringle (16) mentions purpuric spots occurring after the splashing of selenous acid on the forearms. Another patient had a weeping, papulopustular eruption on the flexor surfaces of the arms after contact with selenium fumes. Two girls developed paronychia after contact with dry selenium dioxide.

It has been found that the toxicity of selenium is unique in that selenium from different

* From the Department of Dermatology and Syphilology (J. Fred Mullins, M.D., Director) and the Tissue Culture Laboratory (Charles M. Pomerat, Ph.D., Director), The University of Texas Medical Branch, Galveston, Texas.

Received for publication August 30, 1954.

sources is not equally toxic. Selenate is more harmful than the selenite, which in turn is more noxious than selenide. Metallic selenium is least toxic of all. In addition Moxon (11) has found that the method of administration is important. Orally the product is much less toxic for mice than by subcutaneous injection. The response will differ in different species of animal being tested to contact with selenium compounds, and there is even a difference in the susceptibility of different tissues. Age of the test animal must also be considered since the adult individual is less readily affected than the young.

The mode of action of selenium in the body is as much in dispute as the toxicity. There are several theories for which there seems to be some justification by proof. Most authors, including Painter (14), accept the theory that selenium oxidizes sulfhydryl compounds forming the disulfide and an unstable RS—Se—RS complex. This may inhibit certain enzymatic reactions which depend on reversible sulfhydryl $\rightleftarrows$ disulfide changes.

Hopkins and Morgan (6) have shown that the succinic dehydrogenase of the blood is decreased in rats fed Na_2SeO_3 and believe the toxic effect may be on this basis. McConnel and Cooper (9) hypothesize that selenium displaces sulfur in various amino-acids and so makes them unavailable for use.

Even though the exact mode of action and toxicity of selenium is still in dispute, Slinger (24) investigated a 2½% suspension of selenium disulfide under the trade name of Selsun Suspension. He was unable to demonstrate any absorption as indicated by urinary excretion even when the shampoo was used daily for as long as 19 days on eczematous areas of atopic dermatitis. In addition he was unable to demonstrate any toxicity or sensitivity to the Selsun or to any of its individual components. Both Slinger (24) and Slepyan (23) reported control of symptoms in more than 80% of cases of scaling dermatitis of the scalp.

Because of the efficacy of this product and its increasingly widespread use, it was deemed advisable to test its toxicity by tissue culture methods which had shown close correlation to toxicity of antibiotics (4). Selenium had been previously studied in tissue culture by various authors. A. H. Roffo in 1925 (19) found that potassium selenate inhibited cellular division in dilutions of less than 1:10,000 in normal tissue and in dilutions of 1:100,000 in neoplastic tissue. In 1939 (18) he reported a slight inhibitory effect of metallic selenium on sarcoma fibroblasts. Nita (13), working on fibroblastic outgrowths, found the selenium in low concentrations increased growth but with increase in concentration inhibited growth. Ruffilli (22) found that cultures of chick myocardium were inhibited by 0.01% colloidal selenium, 0.001% selinuria and by 0.0033% selenium dioxide.

METHOD

From the previous work which had been done on selenium compounds, we had some idea where the toxicity would lie; however, no work had actually ever been done using selenium disulfide. Although we wanted to work primarily with human tissues, a preliminary titration was set up using 19-day-old chick spleen to determine the approximate least injurious dose (LID), the smallest quantity to show the first effects of injury, and the minimal inhibitory dose (MID), the smallest quantity to produce complete inhibition of growth. In addition, a duplicate titration was run on the vehicle alone containing all the essential ingredients except the selenium disulfide.

The method used was similar to that of Everett (4), in which the essential steps are as follows:

1. The spleens were removed aseptically from 19-day-old chick embryos and divided into cubes of approximately 1 mm. in each dimension.
2. Each fragment was then embedded in a clot composed of one drop of embryonic extract juice (EEJ) and one drop of cockerel plasma on a sterile ⅞″ #1 square cover slip.

TABLE I

Preliminary titration using 19 day old chick spleen

	Control	1:1,000,000	1:100,000	1:10,000	1:1000	1:100	1:10
				Vehicle			
4+	0	0	0	0	0	0	0
3+	37.5%	0	12.5%	37.5%	0	0	0
2+	25.0%	87.5%	75.0%	25.0%	25.0%	0	0
1+	37.5%	12.5%	12.5%	37.5%	37.5%	0	0
±	0	0	0	0	37.5%	100%	0
No growth	0	0	0	0	0	0	100%
				Selsun			
4+		0	0	0	0	0	
3+		0	0	0	0	0	0
2+		75%	75%	60%	0	0	0
1+		25%	25%	40%	20%	0	0
±		0	0	0	80%	100%	0
No growth		0	0	0	0	0	100%

3. The selenium disulfide suspension or vehicle had been added to the embryonic extract in dilutions ranging from 1:1,000,000 to 1:10 with appropriate drug free controls.
4. The cover slip was inverted on a medium sized hanging drop slide after the clot had set and sealed with paraffin wax and incubated at 37° C. for 24 hours.

TABLE II

Titration using human skin

	Control	1:100,000	1:10,000	1:1000	1:100
			Vehicle		
4+	0	0	0	0	0
3+	0	0	0	0	0
2+	70%	65%	0	0	0
1+	30%	5%	20%	0	0
±	0	30%	80%	40%	0
No growth	0	0	0	60%	100%
			Selsun		
4+		0	0	0	
3+		0	0	0	0
2+		70%	0	0	0
1+		30%	80%	0	0
±		0	20%	20%	0
No growth		0	0	80%	100%

TABLE III

Comparison of MID and LID

	No. of Culture	LID	MID
	Chick Spleen		
Control	30		
Vehicle	50	1:1000	1:10
Selsun	100	1:1000	1:10
	Human Skin		
Control	40		
Vehicle	110	1:10,000	1:100
Selsun	120	1:10,000	1:100

At the end of 24 hours, the chick spleen had reached its maximum outgrowth, and the cultures were read on a microscope using a 16 mm. objective and a ×10 ocular. Fifty cultures using the vehicle and 100 cultures using Selsun were set up. These were graded as 1+, 2+, 3+, or 4+ depending on whether the outgrowth extended ¼, ½, ¾ or entirely across the field respectively. They were graded as ± if there was some outgrowth but less than ¼ of the field was covered.

The experiment was then repeated using human skin (table II). This was cut by means of a microtome on a remnant of skin left over after a grafting procedure. The method used was similar to that used for the chick spleen with the following exceptions:

1. The skin was cut into squares approximately 2 mm. x 2 mm.
2. One gamma/cc. of neomycin sulfate was added to the plasma as a bacterial inhibitor.
3. After the clot had set, four drops of ascitic fluid from a patient with abdominal carcinomatosis was added as nutrient material.
4. Cultures were examined every day or two until the seventh day, at which time maximum outgrowth was obtained.

One hundred and ten cultures using the vehicle and 120 cultures using Selsun were set up. Representative sections were fixed in methyl alcohol and stained according to the technic of Jacobson for permanent records.

Some difficulty was encountered in reading the more concentrated dilutions in that the material being a suspension tended to obscure the cell outlines; however, by focusing up and down with the objective it was found that the particles of the suspension were at a slightly different level than the cells of the outgrowth in most cases and so probably did not give rise to a significant error.

DISCUSSION

The toxicity of selenium disulfide suspension on chick spleen growing out in tissue culture is of the same magnitude as the toxicity on human skin when tested

in the manner described. In this way an accurate estimate of the best range of dilutions to use was determined within 24 hours by using the faster growing, more easily cultured chick spleen as a preliminary titration.

According to our interpretation of the experimental data, there is no appreciable difference in the toxicity of Selsun as compared to the suspending vehicle alone. It must be remembered, however, that this method would determine only toxicity to the skin or primary irritation capacity. This study does not attempt to evaluate any allergic response or such toxic reactions which might occur in other organs if selenium disulfide penetrates the skin.

Some of the effects seen might be due to the other ingredients in the formula, which is as follows:

	%
Selenium disulfide	2.5
A Nacconol (surface active agent)	17.0
Inert and stabilizing ingredients	5.2
Water qs. ad	100.0

In our clinical experience the sensitization index must be very low in any event since we have not encountered a single case of sensitivity which could be directly related to selenium disulfide in four years of frequent use in clinic and private practice. To date we have been unable to find a single case of sensitivity reported in the literature.

SUMMARY

1. The uses of selenium in industry and medicine, which are more extensive than generally considered, have been reviewed.

2. The toxicity of selenium-containing compounds for animals and humans with relation to selenium disulfide suspension as a therapeutic agent for seborrheic dermatitis has been discussed.

3. An experiment using tissue culture technics first on chick spleen, then on human skin, to show that 2.5% selenium disulfide suspension is no more toxic than the vehicle alone has been described.

REFERENCES

1. Buchan, R. F.: Industrial selenosis. Occup. Med., **3:** 439–456, 1947.
2. Dudley, H. C.: Toxicology of selenium II. The urinary excretion of selenium. Am. J. Hyg., **23:** 181–186, 1936.
3. Dudley, H. C.: Pub. Health Rep., **53:** 94–9, 1938.
4. Everett, E. T., Pomerat, C. M., Hu, F. N. and Livingood, C. S.: Tissue culture studies on human skin I. Texas Rep. Biol. & Med., **9:** 281–291, 1951.
5. Hall, R. H. et al: Preliminary observations on toxicity of elemental selenium. A.M.A. Arch. Indust. Hyg., **4:** 458–464, 1951.
6. Hopkins, F. G. and Morgan, E. J.: The influence of thiol-groups in the activity of dehydrogenases. Biochem. J., **32:** 611–620, 1948.
7. Klug, H. L.: The in vivo inhibition of succinic dehydrogenase by selenium and its release by arsenic. Arch. Biochem., **28:** 253–259, 1950.
8. Lemley, R. E. and Merryman, M. P.: Selenium poisoning in the human. Lancet, **61:** 435–438, 1941.

9. McConnell, K. P. and Cooper, B. J.: Distribution of selenium in serum protein and red blood cells after subcutaneous injection of sodium selenate containing radioselenium. J. Biol. Chem. **183:** 459–466, 1950.
10. Middleton, J. M.: Selenium burn of the eye. Arch. Ophth., **38:** 806–811, 1947.
11. Moxon, A. L., Paynter, C. R. and Halverson, A. W.: Effect of route of administration of detoxification of selenium by arsenic. J. Pharmacol. & Exper. Therap., **84:** 115–119, 1945.
12. Muehlberger, C. W. and Schrenk, H. H.: The effect of the state of oxidation on the toxicity of certain elements. J. Pharmacol. & Exper. Therap. **33:** 270, 1928.
13. Nita, B.: Folia Pharmacol. Japan, **21:** 336–46, 1936. Cited by Murray, M. R. and Kopech, G.: A Bibliography of the Research in Tissue Culture 1884–1950. New York, Academic Press, Inc., 1953.
14. Painter, Edgar Page: The chemistry and toxicity of selenium compounds with special reference to the selenium problem. Chem. Rev., **28:** 178–213, 1941.
15. Pomerat, C. M.: Cited by Visscher, M. B., ed., Methods in Medical Research, **4:** 260–272. Chicago, The Year Book Publishers, Inc., 1951.
16. Pringle, P.: Occupational dermatitis following exposure to inorganic selenium compounds. Brit. J. Dermat., **54:** 54–58, 1942.
17. Robinson, O. W.: J. Assoc. Off. Agr. Chem., **16:** 423, 1933. Cited by Smith, M. I., Franke, K. W., and Westfall, B. B.: Pub. Health Rep., **51:** 1496–1505, 1936.
18. Roffo, A. H.: Accion Biol a Distancia de los Metales, Inst. Biol. Med. Exp., Buenos Aires, **16(50):** 55–76, 1939.
19. Roffo, A. H.: Bol. Inst. de med. exper. para el estur. y trat. d. cáncer, Buenos Aires, **2:** 847–868, 1925. Cited by Murray, M. R. and Kopech, G.: A Bibliography of the Research in Tissue Culture 1884–1950. New York, Academic Press, Inc., 1953.
20. Rosenfeld, Irene and Beath, O. A.: The influence of various substances on chronic selenium poisoning. J. Pharmacol. & Exper. Therap., **91:** 218–233, 1947.
21. Rosenfeld, Irene and Beath, O. A.: Metabolism of sodium selenate and selenite by the tissues. J. Biol. Chdm., **172:** 333–341, 1948.
22. Ruffilli, D.: Studi Sassuresi, **26:** 95–7, 1948. Cited by Murray, M. R. and Kopech, G.: A Bibliography of the Research in Tissue Culture 1884–1950. New York, Academic Press, Inc., 1953.
23. Slepyan, A. H.: Selenium disulfide suspension in treatment of seborrheic dermatitis of the scalp. Arch. Dermat. & Syph., **65:** 228–229, 1952.
24. Slinger, W. N.: Treatment of seborrheic dermatitis with a shampoo containing selenium disulfide. Arch. Dermat. & Syph., **64:** 41–48, 1951.
25. Smith, M. I., Franke, K. W. and Westfall, B. B.: Pub. Health Rep., **51:** 1496–1505, 1936.
26. Smith, M. I. and Westfall, B. B.: Pub. Health Rep., **52:** 1375–84, 1937.